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Comparative Evaluation of Phenols, Flavonoids and Antioxidant Activity of Flax Seed from Two Locations

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In this study, the defatted seeds of Flax from two locations were extracted with acetone, ethanol and water separately. These extracts were used to evaluate total phenols, flavonoids, mineral content and antioxidant activity by three methods: 2,2'-diphenyl-1-picrylhydrazyl, ferric thiocyanate and β-carotene bleaching method. Both total phenolics and flavonoid content were higher in ethanol extract of Flax seed (Rajmahal hills) (360.00 ± 0.40 mg GAE/g and 185.00 ± 3.29 mg CAE/g of the extract) respectively. According to DPPH method, antioxidant activity of acetone extract of Flax seed (Rajmahal hills) was found to be maximum that is 79.29 ± 0.71 while by FTC method it was maximum for ethanol extract of Flax seed (Rajmahal hills) (71.42 ± 0.37) and by β-carotene bleaching method, water extract of Flax seed (Rajmahal hills) showed maximum antioxidant activity *i.e.* 83.06 ± 0.31.

Keywords: *Linum usitatissimum*, Total phenolics, Flavonoids, Antioxidant activity, Mineral content.

INTRODUCTION

In recent years, a renewed interest has been witnessed on the adoption of crude extracts of plants as pharmaceuticals. By improving the intake of phytochemicals and natural nutrients with antioxidant properties, several diseases can be prevented which are caused due to oxidative stress. Oxidative stress releases free oxygen radicals in the body which can damage membrane lipids, proteins, nucleic acids and carbohydrates and result into many diseases like cancer, cardiovascular diseases, neurodegenerative diseases and inflammation [1-3]. Free radicals as well as reactive oxygen species are highly reactive in nature and react rapidly with adjacent molecules *via* a variety of reactions involving hydrogen abstraction (capturing), electron donation and electron sharing [4]. Hence, these species needs to be scavenged *via* free radical traps such as phenols or by antioxidative enzymes like glutathione peroxidase and glutathione reductase to maintain cellular functions. Many phenolic compounds like flavonoids, tannins and lignan precursors act as potential antioxidants in plant tissues. The free radical scavenging activity of polyphenols arises from their high reactivity as hydrogen or electron donors and from their ability to stabilize and delocalize the unpaired electrons and to chelate transition metal ions [4,5].

Flax (*Linum usitatissimum*) which is commonly known as Flax seed, linseed, alsi, *etc*. belongs to the family Linaceae. It is grown either as an oil crop or as a fibre crop. Flax seed contains 40-50 % oil and its meal is composed of 23-34 % protein, 4 % ash, 5 % viscous fibre (mucilage) and lignin precursors depending upon the cultivar and growing conditions [6]. Since time immemorial, Flax seed is used as one of the most effective cholesterol lowering foods. The seeds of Flax act as analgesic, pectoral, demulcent, laxative and resolvent. It also helps in reducing heart disease and stroke. Flax seed contains several amazing natural compounds including lignans, essential omega-3 fatty acids and dietary fibres [7]. Its potential health benefits are due to its high nutritive value which depends upon cultivar, locality, sowing and year of production. So, the present study was aimed at the comparison of chemical composition and antioxidant activity of different extracts of Flax seed grown at two locations.

EXPERIMENTAL

The commercially available chemicals from Sigma-Aldrich, Qualigens, Merk and Ranbaxy, of high purity, were used for various experimental procedures.

Flax seeds were procured from two regions, one from Hisar (Semi-arid zone) and other from Rajmahal Hills (Humid sub-tropical zone). Flax seeds were first crushed in a grinder to convert into powder form and the powdered seeds were then extracted with hexane, acetone, ethyl alcohol and distilled water, separately. Total phenol, flavonoid, mineral content and

the antioxidant activity of these extracts were then determined using various methods.

Preparation of extracts: 100 g samples of each plant material were extracted separately first with hexane to extract oil from the seeds. The defatted seeds were then extracted separately with acetone, ethyl alcohol and distilled water by refluxing for 6 h and the process repeated three times. The solvent was removed to get extractives. These extracts were filtered and concentrated under reduced pressure and used for estimation of different parameters.

Determination of total phenolics content: The total phenolics were determined by the Folin-Ciocalteu reagent [8] method using gallic acid as standard. The absorbance was measured at 730 nm using Spectronic 20 (Milton Roy Company) spectrophotometer against a blank. The results were expressed as equivalent to milligrams of gallic acid per gram of extract (mg GAE/g).

Determination of flavonoid content: The aluminium chloride colorimetric assay [9] was used. The absorbance was read at 510 nm using UV visible spectrophotometer. Total flavonoid content was expressed as mg catechin equivalents per gram of the extract (mg CAE/g).

Determination of mineral contents: The sample was digested by wet oxidation. 0.2 g of each extract was transferred to a conical flask. 1 mL of perchloric acid and 5mL nitric acid was added, mixed well and kept overnight at room temperature followed by digestion on low temperature 70-80 °C and then at higher temperature until the volume of the solution reduced to about 1 mL. After the digestion of all, the mixture volume was made to 15 mL with distilled water and analyzed by using atomic absorption spectrometer (AAS).

Antioxidant activity

2,2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method: The antioxidant activity of the extracts was evaluated by DPPH free radical scavenging method [10] and the effect of extracts on DPPH radical was estimated. The absorbance of the sample was measured at 517 nm using spectrophotometer Spectronic 20 (Milton Roy Company). The percentage of DPPH, which was scavenged (%DPPH*$_{sc}$) was calculated using:

$$DPPH^{*}_{sc}(\%) = \frac{(A_{cont} - A_{samp})}{A_{cont}} \times 100$$

where A_{cont} is the absorbance of control and A_{samp} is the absorbance of sample.

Ferric thiocyanate (FTC) method: The FTC method [11] was used to evaluate the antioxidant activity of the extract. The colour developed was measured at 500 nm using the spectrophotometer. Antioxidant activity was expressed as:

$$\text{Antioxidant activity } (\%) = \left(1 - \frac{\text{Increase in abs. of sample}}{\text{Increase in abs. of control}}\right) \times 100$$

β-Carotene bleaching method: The β-carotene bleaching method [12] was used to evaluate the antioxidant activity of the extract. The absorbance at 470 nm was recorded with a Spectronic 20 spectrophotometer. The antioxidant activity (AA) was expressed as percentage inhibition relative to the control using the equation:

$$AA(\%) = 100\left(1 - \frac{(A_0 - A_t)}{(A_0^0 - A_t^0)}\right)$$

where A_0 and A_0^0 are the absorbance values measured at zero time of incubation for the test sample and control respectively and A_t and A_t^0 are the corresponding values at the end of the reaction time.

Statistical analysis: The experimental measurements were carried out in triplicate and results were presented as mean of three replicates ± standard deviation. Statistical analysis was carried out using Microsoft Excel 2007.

RESULTS AND DISCUSSION

Yield percentage: The yield of extractable compounds relative to the weight of defatted dried seed material ranged from 4.35 ± 0.17 % (acetone extract) to 21.93 ± 0.23 % (water extract) in Flax seed (Hisar) and 2.56 ± 0.35 % (acetone extract) to 17.77 ± 0.41 % (ethanol extract) in Flax seed (Rajmahal hills) as in Table-1. Flax seed from semi-arid zone showed high per cent yield in all the three extracts as compared to the humid subtropical zone.

Total phenolic content: Phenolics are aromatic secondary plant metabolites are high-level antioxidants because of their ability to scavenge free radicals and active oxygen species such as superoxide free radicals and hydroxyl radicals. It is well known that phenolic substances contribute directly to the antioxidant activity of plant materials. In fact, phenolic compounds exhibit considerable free radical-scavenging activities (through their reactivity as hydrogen-donating or electron-donating agents) and metal ion-chelating properties [13]. Present results showed that the content of total phenols varied from 153.00 ± 1.41 mg GAE/g (acetone extract) to 267.00 ± 4.24 mg GAE/g (water extract) in Flax seed (Hisar) and 219.00 ± 4.23 mg GAE/g (acetone extract) to 360.00 ± 0.40 mg GAE/g (ethanol extract) in Flax seed (Rajmahal hills) as shown in Fig. 1. The difference in the phenolic content in Flax seed from two different locations may be attributed due to their agro-climatic differences; further total phenol content in the Flax seeds from humid sub-tropical zone was higher than semi-arid zone.

Flavonoid content: Flavones and flavonols are the sub-groups of flavonoids. Flavonols are known to act as antioxidant, both as radical scavengers [14] and as metal chelators [15]. The aglycones of these flavonols were reported to be more active than their glycosides [16]. Flavonoids have the ability to scavenge active oxygen radical, superoxide and hydroperoxide by single electron transfer. Superoxide is a

TABLE-1
YIELD PERCENTAGE OF DIFFERENT EXTRACTS OF FLAX SEED OF HISAR AND RAJMAHAL HILLS

Seed samples	Acetonic extract (%)	Ethanolic extract (%)	Water extract (%)
Flax seed (Hisar)	4.35 ± 0.17	18.44 ± 0.36	21.93 ± 0.23
Flax seed (Rajmahal hills)	2.56 ± 0.35	17.77 ± 0.41	16.76 ± 0.16

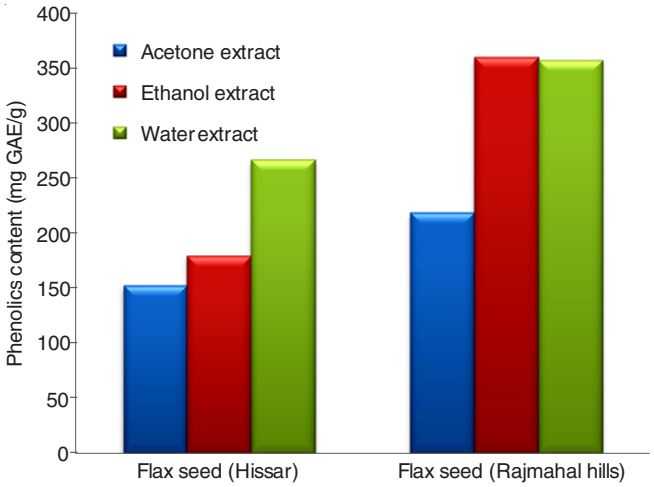

Fig. 1. Total phenolics content of different extracts of Flax seed (Hisar) and Flax seed (Rajmahal hills)

biologically important substance which can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals [17]. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins [18]. In the present study the flavonoid content in Flax seed (Hisar) varied from 62.67 ± 0.23 mg CAE/g (ethanol extract) to 127.0 ± 1.63 mg CAE/g (water extract). In the Flax seed (Rajmahal hills), it varied from 92.00 ± 0.94 mg CAE/g (water extract) to 185.00 ± 3.29 mg CAE/g (ethanol extract) (Fig. 2).

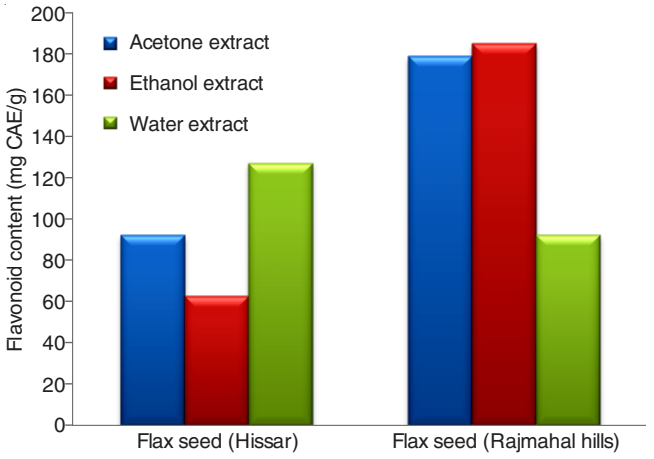

Fig. 2. Flavonoid content of different extracts of Flax seed (Hisar) and Flax seed (Rajmahal hills)

The flavonoid content of the acetonic and ethanolic extract of Flax seed from Rajmahal hills were much higher which might be due to the presence of higher content of methylated and acylated form of phenols and flavonoids as well as its aglycone content. The same sample showed a lower content of flavonoids in the water extract, which also supports the above result as the methylated and acylated form of flavonoids are less soluble in water.

Elemental composition: The concentrations of most of the mineral elements are significantly different among different locations of Flax seed (Table-2). Iron was the most predominant element present in all extracts of Flax seed. The concentration of Fe varies from 254.67 ± 0.27 ppm (ethanol extract) to 283.04 ± 0.16 ppm (water extract) in Flax seed (Hisar) and from 133.77 ± 0.12 ppm (water extract) to 194.02 ± 0.81 ppm (acetone extract) in Flax seed (Rajmahal hills). Among other elements, zinc was present in significant amounts with highest amounts in ethanolic extract of Flax seed (Rajmahal hills). The zinc content varied from 15.00 ± 0.61 ppm (water extract) to 19.50 ± 0.21 ppm (ethanol extract) in Flax seed (Hisar) and from 9.02 ± 0.16 ppm (water extract) to 26.32 ± 0.15 ppm (ethanol extract) in Flax seed (Rajmahal hills). The copper and manganese contents were present in low concentrations. The copper content varied from 2.27 ± 0.16 ppm (acetone extract) to 6.67 ± 0.11 ppm (water extract) in Flax seed (Hisar) and from 1.50 ± 0.20 ppm (water extract) to 3.76 ± 0.14 ppm (ethanol extract) in Flax seed (Rajmahal hills). Similarly, the Mn content varied from 4.86 ± 0.12 ppm (ethanolic extract) to 5.47 ± 0.24 ppm (acetone extract) in Flax seed (Hisar) and from 3.45 ± 0.16 ppm (acetonic extract) to 4.02 ± 0.13 ppm (water extract) in Flax seed (Rajmahal hills). These elements can become highly toxic if they accumulate in biological system. However, their concentrations were much below the toxicity level [19].

Antioxidant activity: Antioxidants affect the process of lipid oxidation at different stages due to differences in their mode of action [20]. Oxidation of lipids is a very complex process resulting in a great variety of oxidation products. Many factors, particularly temperature, light and the presence of initiators (metal enzymes), influence the oxidation process and resulting products. For this reason different methods are needed for monitoring oxidation processes to assess primary or secondary oxidation changes and the efficiency of antioxidants. The results obtained by different methods can also differ distinctly, as they involve different conditions, reaction phases and reaction systems. Therefore, to obtain more comprehensive information on antioxidants, the present study is aimed at the evaluation of the antioxidant activity of different extracts of Flax seed (Hisar) and (Rajmahal hills) by using three testing methods.

I. 2,2'-Diphenyl-1-picrylhydrazyl radical (DPPH) method
II. Ferric thiocyanate method
III. β-Carotene bleaching method

TABLE-2
MINERALS CONTENT OF DIFFERENT EXTRACTS OF FLAX SEEDS OF HISAR AND RAJMAHAL HILLS

Elements	Acetone extracts (ppm)		Ethanol extracts (ppm)		Water extracts (ppm)	
	Flax seed (Hisar)	Flax seed (Rajmahal hills)	Flax seed (Hisar)	Flax seed (Rajmahal hills)	Flax seed (Hisar)	Flax seed (Rajmahal hills)
Cu	2.27 ± 0.16	1.55 ± 0.34	3.82 ± 0.66	3.76 ± 0.14	6.67 ± 0.11	1.50 ± 0.20
Fe	262.20 ± 0.24	194.02 ± 0.81	254.67 ± 0.27	141.62 ± 0.10	283.04 ± 0.16	133.77 ± 0.12
Mn	5.47 ± 0.24	3.45 ± 0.16	4.86 ± 0.12	3.67 ± 0.17	5.22 ± 0.14	4.02 ± 0.13
Zn	15.83 ± 0.23	10.57 ± 0.32	19.50 ± 0.21	26.32 ± 0.15	15.00 ± 0.61	9.02 ± 0.16

Antioxidant activity by DPPH method: Various radicals formed during lipid oxidation are among the main causes for oxidative damage to human health [21]. Antioxidants can exercise their protective function by scavenging free radicals, which are the main propagators of lipid oxidation.

2,2'-Diphenyl-1-picrylhydrazyl radical is one of the few stable and commercially available organic nitrogen radical ($DPPH^{\bullet}$), often used in evaluation of radical scavenging activity of antioxidants-natural and synthetic pure compounds [22,23], plant extracts [24,25] and foods [26]. Alcoholic solutions of $DPPH^{\bullet}$ have a characteristic absorption maximum at 517 nm. When an electron or hydrogen atom donating antioxidant (AH) is added to $DPPH^{\bullet}$ a decrease in absorbance at 517 nm takes place due to the formation of the non-radical form DPPH-H which does not absorb at 517 nm. Originally, it was monitored by ESR spectroscopy and relied on the signal intensity of $DPPH^{\bullet}$ being inversely related to the antioxidant concentration and the reaction time. More recently, this reaction has been measured by the de-colouration assay where the decrease in absorbance at 517 nm produced by the addition of the anti-oxidant to the $DPPH^{\bullet}$ in methanol or ethanol is measured.

$$DPPH^{\bullet} + AH \longrightarrow DPPH\text{-}H + A^{\bullet}$$

All the above described extracts were screened for radical scavenging activity against $DPPH^{\bullet}$. The most active type of extracts was acetone extracts (Figs. 3 and 4). This might be due to the presence of lower phenols and non-glycosylated flavonols which are generally more soluble in acetone. This suggests that the extract may contain higher concentrations of active compounds needed in the reaction of DPPH scavenging. In general, the radical scavenging activities of water extracts were much lower and strongly influenced by the plant sample origin. The highly polar solvent extracts were not the most active in the $DPPH^{\bullet}$ test system. This fact could mean that either their components do not possess, good hydrogen donating properties or some kinetic factors influenced their reaction with the radical, or their components interfere with the radical scavenging process. The radical scavenging activity of a particular antioxidant depends on structure as well as on the type of reaction kinetics [27].

Antioxidant activity by ferric thiocyanate method: Real food systems generally consist of multiple phases in which lipid and water coexists with some emulsifier. Hence an antioxidant assay using a heterogeneous system such as an oil-in-water emulsion is required. Autoxidation of linoleic acid in ethanol buffer is one of the model systems for such evaluation, satisfying the above conditions [28]. The linoleic acid emulsion system/thiocyanate method has been used here for evaluation under the above conditions. During peroxidation of linoleic acid at 37 °C in an incubator, the absorbance values increased owing to the oxidation products, which react to form ferric thiocyanate, the colour of red blood [29]. Antioxidants can hinder the oxidation and, consequently, the increase in absorbance will be less. The antioxidant activity exhibited by acetone, ethanol and water extracts of Flax seed (Hisar) were 33.35 ± 0.81 %, 62.49 ± 1.63 % and 60.09 ± 0.72 % at the concentration of 0.5 mg/mL of the extract respectively. The corresponding values of Flax seed (Rajmahal hills) at the same concentration were 50.00 ± 0.82 %, 71.42 ± 0.37 % and 55.56 ± 0.45 % respectively (Figs. 3 and 4). The antioxidant activity of the extracts by thiocyanate method supports the results of the β-carotene bleaching method.

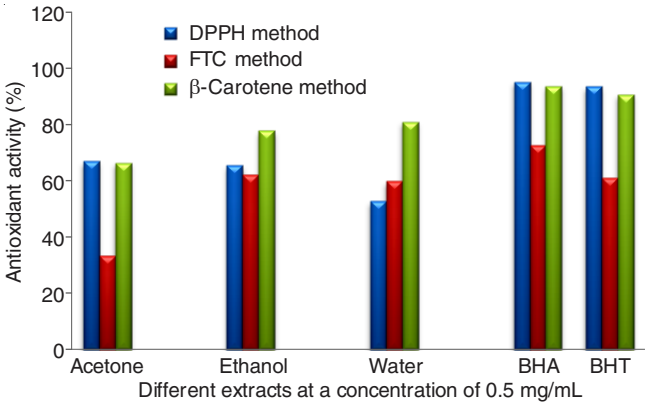

Fig. 3. Antioxidant activity of different extracts of Flax seed (Hisar) by DPPH, FTC and β-carotene bleaching method

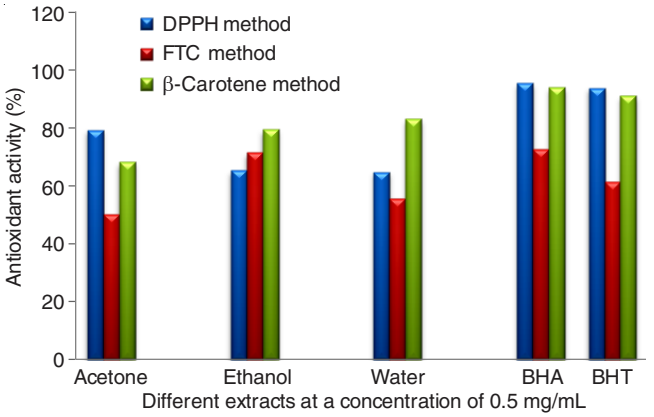

Fig. 4. Antioxidant activity of different extracts of Flax seed (Rajmahal hills) by DPPH, FTC and β-carotene bleaching method

Antioxidant activity by β-carotene bleaching method: In the β-carotene bleaching method, the antioxidant activity of carotenoids is based on the radical adducts of carotenoid with free radicals from linoleic acid. The linoleic acid free radical attacks the highly unsaturated β-carotene models. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system [29]. In this way the antioxidant activity of various added substances can be monitored. Our results showed that the antioxidant activity of Flax seed (Hisar) varied from 66.63 ± 0.30 % (acetone extract) to 81.25 ± 0.16 % (water extract) and from 68.10 ± 0.15 % (acetone extract) to 83.06 ± 0.31 % (water extract) in Flax seed (Rajmahal hills) at the concentration of 0.5 mg/mL of the extract (Figs. 3 and 4). The highly polar solvent extracts of Flax seed were found to have higher activity at high concentration. Additionally it seems that antioxidant activity of all the extracts showed different value from the extracts of the different polar solvent.

REFERENCES

1. B.N. Ames, *Science*, **221**, 1256 (1983).
2. N. Dasgupta and B. De, *Food Chem.*, **101**, 471 (2007).
3. Y. Sun, *Free Radic. Biol. Med.*, **8**, 583 (1990).
4. J.M. McCord, *Am. J. Med. Sci.*, **108**, 652 (2000).

5. C.A. Rice-Evans, N.J. Miller and G. Paganga, *Trends Plant Sci.*, **2**, 152 (1997).
6. A.D. Muir and N.D. Wescott, Flax: The Genus Linum, Taylor and Francis, London (2003).
7. S.C. Cunnane, S. Ganguli, C. Menard, A.C. Liede, M.J. Hamadeh, Z.-Y. Chen, T.M.S. Wolever and D.J.A. Jenkins, *Br. J. Nutr.*, **69**, 443 (1993).
8. V.L. Singleton and J.A. Rossi, *Am. J. Enol. Vitic.*, **16**, 144 (1965).
9. D. Marinova, F. Ribarova and M. Atanassova, *J. Univ. Chem. Technol. Metallurgy*, **40**, 255 (2005).
10. T. Hatano, H. Kagawa, T. Yasuhara and T. Okuda, *Chem. Pharm. Bull. (Tokyo)*, **36**, 2090 (1988).
11. H. Kikuzaki and N. Nakatani, *J. Food Sci.*, **58**, 1407 (1993).
12. M.E. Hidalgo, E. Fernandez, W. Quilhot and E. Lissi, *Phytochemistry*, **37**, 1585 (1994).
13. A. Rice-Evans, N.J. Miller and G. Paganga, *Free Radic. Biol. Med.*, **20**, 933 (1996).
14. W. Bors and M. Saran, *Free Radic. Res. Commun.*, **2**, 289 (1987).
15. I.B. Afanas'ev, A.I. Dcrozhko, A.V. Brodskii, V.A. Kostyuk and A.I. Potapovitch, *Biochem. Pharmacol.*, **38**, 1763 (1989).
16. A. Hopia and M. Heinonen, *J. Am. Oil Chem. Soc.*, **76**, 139 (1999).
17. M. Korycka-Dahl and M. Richardson, *J. Dairy Sci.*, **61**, 400 (1978).
18. M. Grootveld and R. Jain, *Free Radic. Res. Commun.*, **6**, 271 (1989).
19. WHO, WHO Technical Report, World Health Organization, Geneva (1989).
20. R.A. Larson, Naturally Occurring Antioxidants, Lewis Publishers, New York (1997).
21. G. Williams, Antioxidants, Princeton Scientific Publishing Co-operation Inclusive, Princeton (1993).
22. W. Brand-Williams, M.E. Cuvelier and C. Berset, *Lebenson. Wiss. Technol.*, **28**, 25 (1995).
23. A. von Gadow, E. Joubert and C.F. Hansmann, *Agric. Food Chem.*, **45**, 632 (1997).
24. I.I. Koleva, T.A. van Beek, J.P.H. Linssen, A. Groot and L.N. Evstatieva, *Phytochem. Anal.*, **3**, 8 (2002).
25. G.C. Yen and P.D. Duh, *J. Agric. Food Chem.*, **42**, 629 (1994).
26. T. Yamaguchi, H. Takamura, T. Matoba and J. Terao, *Biosci. Biotechnol. Biochem.*, **62**, 1201 (1998).
27. V. Bondet, W. Brand Williams and C. Berset, *Lebenson. Wiss. Technol.*, **30**, 609 (1997).
28. T. Osawa and M.A. Namiki, *Agric. Biol. Chem.*, **45**, 735 (1981).
29. G.K. Jayaprakasha, R.P. Singh and K.K. Sakariah, *Food Chem.*, **73**, 285 (2001).